

Supplementary data for:

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TbVps34, the Trypanosome Orthologue of Vps34, Is Required for Golgi Complex Segregation*[§]

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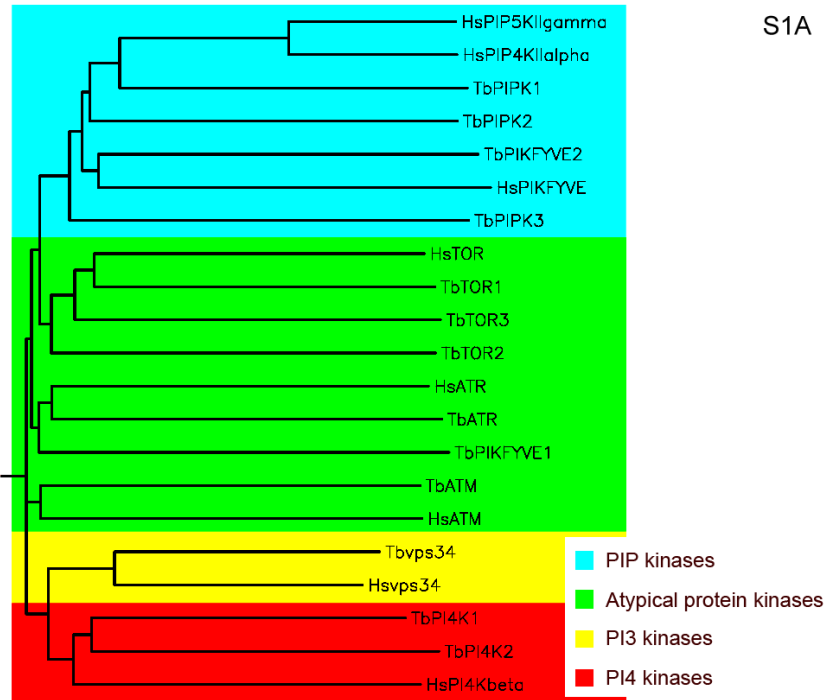
Supplementary figure legends

Figure S1: TbVps34 is a Class III PI 3-kinase. A. Phylogenetic reconstruction of PI kinase domain proteins in the *T. brucei* genome generated with Clustal W alignments with human orthologues using 1000 bootstraps. PI 3-kinases, PI 4-kinases, PIP kinases and atypical protein kinases fall into distinct subgroups in accordance with predicted activity. B. Clustal W alignment comparing the catalytic domains of Class III PI 3-kinases from *Homo sapiens* (Hs), *Arabidopsis thaliana* (At) and *Saccharomyces cerevisiae* (Sc) with the *vps34* orthologue from *T. brucei*. The alignment has been shaded using TeXshade software. Colour coding: black, identical in all four sequences; dark grey, conserved between two or three sequences; light grey, conservative substitution; "." indicates a gap introduced into the alignment. Residue numbers are shown relative to the *T. brucei* sequence.

Figure S2: TbVPS34 RNAi alters VSG localisation. p2T7TbVPS34 cells were incubated for 24hr in the absence (-TET) and presence (+TET) of 1 µg ml⁻¹ tetracycline. Cells were fixed with 4% paraformaldehyde for 1hr at 4°C and permeabilised with 0.1% Triton-x-100. Slides were blocked with 10% goat serum in PBS, then incubated for 1hr with rabbit anti-VSG 221 antiserum. Slides were washed and incubated with Texas Red-labelled goat anti-rabbit IgG (Molecular Probes). Slides were counterstained with DAPI (blue) to visualise the nucleus and kinetoplast.

Figure S3. Inhibition of Golgi segregation is not seen in RNAi of a trypanosomal PI 4-kinase. A fragment of the predicted PI 4-kinase TbPI4K1 (GeneDb Tb927.4.1140, accession number AAX79183) was amplified by PCR and inserted into p2T7Ti and transfected into BSF as described. Selected cells were transfected into pHD1034.RFPGRASP and induced for RNAi with 1 µg ml⁻¹ tetracycline for 24hr. Images show cells at different stages of the cell cycle fixed with 4% paraformaldehyde and stained with DAPI (blue). In this case, multiple GRASP containing compartments are clearly present in most mitotic cells and no defect in segregation is apparent. In addition no evidence for expansion of the Golgi was observed by EM (data not shown).

S1A



MATNEDTVAYTLKEENCOTLRGAPPSEFLAANDHEPFLMLICAVGVARACRRANREET
 10 20 30 40 50 60
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

SSRCNSWIAEIKKPEPTVIEALWIDGATFCQPMV
 70 80 90 100 110 120
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

RRLKPTPPCYTDELKISSYVDITLASHNNTLVVIVSVCCTFCIGGA
 130 140 150 160 170 180
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

TLLRNSKMGKQKQKREIKGKEDASDFSTPECKVP...RERGKELPR...EKKIMNK
 190 200 210 220 230 240
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

YERGQISDM...DRMLKSDIT...EKGESVIA...HCRSHLEWIDE...
 250 260 270 280 290 300
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

GS...FRRWVFQSCAMLF...TAPIGST...WFTVWMDITGKTVSEWQLKARSIDRITL
 310 320 330 340 350 360
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

DRDLRPSNRKSGTORVLRKPT...RTSGDDEQLLKRERSMSERRALKKFKRQVMS
 370 380 390 400 410 420
 AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p

430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580 590 600
 AVps34p AVps34p AVps34p AVps34p AVps34p AVps34p AVps34p AVps34p AVps34p AVps34p
 hVPS34p hVPS34p hVPS34p hVPS34p hVPS34p hVPS34p hVPS34p hVPS34p hVPS34p hVPS34p
 ScVps34p ScVps34p ScVps34p ScVps34p ScVps34p ScVps34p ScVps34p ScVps34p ScVps34p ScVps34p
 TdVps34p TdVps34p TdVps34p TdVps34p TdVps34p TdVps34p TdVps34p TdVps34p TdVps34p TdVps34p

610 620 630 640 650 660 670 680 690 700 710 720 730 740 750 760 770 780 790 800 810 820 830 840
 AVps34p
 hVPS34p
 ScVps34p
 TdVps34p

850 860 870 880 890 900 910 920 930 940 950 960 970 980 990 1000
 AVps34p
 hVPS34p
 ScVps34p
 TdVps34p

S1B

X non conserved
 X similar
 X conserved
 X all match

